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DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP, IV

THE BEHAVIOR OF B. PARATYPHOSUS A AND B. PARATYPHOSUS B IN MILK

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It is now fairly definitely established that freshly isolated paratyphoid A strains slowly produce alkali in milk, and that alkali formation can always be observed when the incubation period is sufficiently prolonged.[1] On the basis of these results it is manifestly incorrect to speak of "permanent acidity" in litmus milk as a distinguishing characteristic of the paratyphoid A type. On the other hand, there is substantial agreement that the paratyphoid B strains produce alkali in milk noticeably earlier than the A strains. Gradations and variations in both types occur, especially in strains that have been for some time under artificial cultivation, but in general the distinction is fairly sharp. If a large series of strains be compared in litmus milk between the 4th and 8th days, it will be very rare indeed that the B. paratyphosus A strains cannot be separated readily from B. paratyphosus B, B. suipestifer and B. enteritidis. In the course of examination of between 200 and 300 strains from many sources, I have found but one strain that could not be so distinguished. This was a paratyphoid B strain (No. 221),[2] typical in all other respects, which does not begin to show alkalinity until about the 10th day and so overlaps some of the more rapid alkali-producing A strains, as for example two or three kindly sent me by Dr. Krumwiede.

In order to determine whether the different behavior of the A and B types in milk is connected with the rate of multiplication of these organisms, I have plated out a series of cultures at different ages.

Erlenmeyer flasks of 100 c c capacity and containing 50 c c of fresh certified milk sterilized in the Arnold were inoculated respectively with a number of strains of definitely ascertained characters. Counts were

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[1] Bradley: Jour. and Proc. Roy. Soc. N. S. Wales, 1912, 46, p. 74. Krumwiede, Pratt and Kohn: Jour. Med. Research, 1916, 35, p. 52. Hadley: Jour. Bacteriol., 1917, 2, p. 263. Jordan: Jour. Infect. Dis., 1917, 20, p. 457.

[2] Jordan, E. O.: Jour. Infect. Dis., 1917, 20, p. 457.

made on agar plates after the milk had been incubated at 37 C. Table 1 shows a representative series. The figures are averages of counts of duplicate plates.

TABLE 1

COUNTS TAKEN IN A REPRESENTATIVE SERIES OF STRAINS. MILK AT 37 C.

	No. of Strain[2]	Initial Number Per Cc	After 3 Days	After 7 Days
Para B strains...	12	33,000	730,000,000	560,000,000
	185	26,000	355,000,000	320,000,000
	210	156,000	590,000,000	570,000,000
Suipestifer strains	63	94,000	391,000,000	160,000,000
	167	4,000	470,000,000	318,000,000
	234	589,000	630,000,000	270,000,000
Para A strains...	4	34,000	30,000,000	108,000,000
	158	63,000	23,000,000	83,000,000
	188	12,000	37,000,000	120,000,000

In another series two sets of flasks inoculated with Para B and Para A strains gave the counts described in Table 2.

TABLE 2

COUNTS MADE OF THE STRAINS IN TWO SETS OF FLASKS. MILK AT 37 C.

	Initial Number	After 5 Days	After 15 Days
2 Para B strains (12, 185) 2 flasks each. Average 4 plates...	98,000	830,000,000	103,000,000
2 Para A strains (198, 212) 2 flasks each. Average 4 plates...	90,000	69,000,000	250,000,000

Even when very heavy initial inoculations are made, some difference is apparent, as shown in the series given in Table 3.

TABLE 3

SHOWING THE GRADATIONS WHEN HEAVY INITIAL INOCULATIONS WERE USED. MILK AT 37 C.

	Initial Number	After 3 Days	After 10 Days
4 Para B strains (12, 179, 180, 222) 2 flasks each	14,700,000	942,000,000	432,000,000
3 B. suipestifer strains (63, 118, 167) 2 flasks each	14,900,000	593,000,000	262,000,000
3 B. enteritidis strains (52, 206, 228) 2 flasks each	26,300,000	566,000,000	308,000,000
3 Para A strains (158, 198, 212) 2 flasks each	11,500,000	270,000,000	418,000,000

The multiplication of the Para A strains in milk is generally not as rapid and the numbers do not reach as high a point as is the case with the other members of the group. In this way nearly all the strains described in an earlier paper have been subjected to trial, always with the same result.

On the basis of a large number of counts, into the details of which it does not seem necessary to go, the statement seems warranted that within the first five days after inoculation of approximately equal numbers, the number of Para A bacilli in milk is less than one-half or one-third and often less than one-tenth of the number of Para B bacilli. Connected with this is the fact that the numbers of Para A bacilli generally show an increase between the 3rd and 7th or 3rd and 10th day counts while the B strains show a decrease.

One further interesting observation may be noted. I have already referred to the relatively slow production of alkali by Strain 221. This is connected with a relatively slow multiplication as shown in the series noted in Table 4.

TABLE 4

COMPARISON OF THE RATE OF MULTIPLICATION OF SEVERAL STRAINS

	Initial Number	After 3 Days	After 7 Days
3 Para B strains (12, 185, 210)	72,000	558,000,000	466,000,000
Strain 221	441,000	210,000,000	160,000,000
3 Para A strains (9, 158, 219)......	38,000	31,000,000	76,000,000

TABLE 5

SHOWING THE RELATIVE MULTIPLICATIONS OF PARATYPHOSUS A AND B STRAINS. NEUTRAL MEAT EXTRACT PEPTONE BROTH AT 37 C. USED AS NUTRIENT

	Initial Number Per Cc	After 3 Days	After 7 Days
Para B strain 12.................	9,400	209,000,000	140,000,000
Para B strain 180................	14,000	540,000,000	105,000,000
Para A strain 158................	7,800	14,000,000	70,000,000
Para A strain 219................	9,600	81,000,000	90,000,000

In rate and amount of multiplication in milk, therefore, No. 221 occupies an intermediate position between the common types of paratyphoid A and paratyphoid B bacilli. This corresponds exactly with its relatively tardy alkali production.

The Para A strains also appear to multiply in nutrient broth (Erlenmeyer flasks of 50 c c) more slowly than the Para B strains (Tables 5 and 6).

Flasks of nutrient broth incubated at 20 C. instead of 37 showed a similar difference in the rate of multiplication between the A and B

TABLE 6

COMPARISON OF THE RAPIDITY OF THE MULTIPLICATION OF STRAINS THE NUTRIENT BROTH WAS NEUTRAL MEAT EXTRACT PEPTONE AT 37 C.

	Initial Number Per Cc	After 24 Hours	After 48 Hours	After 72 Hours
Para B strains (5, 8, 299) Average .... (2 plates each flask)	19,200	540,000,000	430,000,000	340,000,000
Para A strains (3, 9, 131) Average .... (2 plates each flask)	11,500	123,000,000	133,000,000	108,000,000

types although the total figures reached were not so great. The addition of 0.1% dextrose to the nutrient broth did not noticeably change the numerical relations of the two types. The rate of multiplication of the Para A strains was still less rapid than that of the B strains.

The differences between the Para A and Para B types in rapidity of alkali formation in milk seem from these facts to be largely, if not altogether, a numerical relation due to gradations in the amount and rate of multiplication.